Switching ON Fetal B Lymphopoiesis

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Switching ON Fetal B Lymphopoiesis

Trine Ahn Kristiansen

LUND UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
to be defended on May 24th 2018 at 9AM in Segerfalk lecture hall, BMC A10,
Lund, Sweden.

Faculty opponent
Professor Ana Cumano
B-1a cells are innate-like lymphocytes that develop primarily during fetal and neonatal life, whereas adult bone marrow (BM) hematopoietic stem cells (HSCs) preferentially give rise to follicular B-2 cells. Functioning at the interface of the innate and adaptive immune systems, B-1a cells provide a non-redundant first line of defense prior to the temporally delayed establishment of a B-2 cell response. The underlying causes for the developmental attenuation in B-1a potential remain poorly resolved. HSCs undergo a functional switch in neonatal mice hallmarked by a decrease in self-renewing divisions and entry into quiescence. The timing of this switch around 3 weeks of age correlates with the change in B cell output from B-1a potent to predominantly B-2 restricted. We hypothesized that the cellular basis for this developmental attenuation in B-1a cell output is a consequence of a shift in stem cell state during ontogeny. Using cellular barcoding for in vivo single-cell resolution analyses, we found that fetal liver definitive HSCs gave rise to both B-1a and B-2 cells. To directly assess whether a developmental shift in HSC state can lead to a selective loss in B-1a potential on a per cell basis, we performed longitudinal comparison of repopulation potential by following barcoded founder cells across serial transplantations. Whereas B-1a potential diminished over time, B-2 output was maintained. B-1a potential could be reinitiated in a subset of adult HSCs by ectopic expression of the RNA binding protein LIN28B, a key regulator of fetal hematopoiesis. This coincided with the clonal reversal to a fetal-like elevated self-renewal and repopulation potential. These results anchor the attenuation of B-1a cell output to fetal HSC behavior and demonstrate that the developmental decline in regenerative potential represents a reversible HSC state. While these data made clear that developmentally restricted hematopoietic origins cannot fully account for the postnatal decline in B-1a output, the underlying mechanism for the positive selection and output of B-1a cells remains elusive. Recent studies showed that ectopic expression of Lin28b in adult pro-B cells was sufficient to potentiate fetal-like B-1a cell output. This led us to next hypothesize that Lin28b may play an important role during the latter part of B lymphopoiesis to potentiate the positive selection of B-1a cells early in life. We showed that CD5 levels of B-1 cells are developmentally set in the immature B cell stage and correlates with self-reactivity. Genetic perturbation studies show that Lin28b is necessary and sufficient for efficient positive selection of B-1a cells and potentiates neonatal immature B cell CD5 expression in a dose dependent fashion. Importantly, our results uncouple positive selection from specific B cell receptor identities, implicating the heterochronic RNA-binding protein LIN28b as the missing link that regulates the developmental attenuation in B-1a cell output through relaxing the permissiveness of B cell selection. Our findings shed light on the unique ability of B-1a cells to escape tolerance and undergo T cell like positive selection. Finally, with ongoing investigations of developmental changes in chromatin accessibility between fetal and adult HSCs we have started to dissect the layers in regulation of a fetal HSC state. Interestingly, we find that regulation of the fetal HSC transcriptome relies more on a post-transcriptional layer compared to adult HSCs. This is consistent with the fetal specific expression pattern of the post-transcriptional regulator Lin28b. Collectively this thesis work has elucidated fetal HSC state and Lin28b associated mechanisms in the attenuation of B-1a cell output during the transition from fetal to adult B lymphopoiesis.

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Faculty of Medicine
Department of Molecular Hematology

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Lund 2018
To my family
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Original Papers and Manuscripts

Papers included in this thesis

Paper I
Cellular barcoding links B-1a B cell potential to a fetal hematopoietic stem cell state

Paper II
Lin28b potentiates positive selection of immature B cells early in life
Manuscript in preparation. 2018

Paper III
Resolving developmental changes in transcriptional and posttranscriptional gene regulation during early hematopoiesis
Manuscript in preparation. 2018

Paper IV
Lentiviral barcode labeling and transplantation of fetal liver hematopoietic stem and progenitor cells
Kristiansen TA, Doyle A, Yuan J
Bio-protocol volume 7, Iss 8, 2017
Published papers not included in this thesis

*Systems-wide analysis of BCR signalosomes and downstream phosphorylation and ubiquitylation*
Molecular Systems Biology, 11, 810, 2015

*Clonal reversal of ageing-associated stem cell lineage bias via a pluripotent intermediate*
Nature Communications, 8, 14533, 2017

*Comprehensive Proteomic Characterization of Ontogenic Changes in Hematopoietic Stem and Progenitor Cells*
Cell Reports, Volume 21, Issue 11, p3285-3297, 2017

*The influence of developmental timing on B cell diversity*
Kristiansen TA*, Vanhee S*, Yuan J
Current Opinion in Immunology, Volume 51, Pages 7-13, 2018 Review
*equal contribution*
Preface

“No man ever steps into the same river twice”

Heraclitus

…for it is not the same river and he is not the same man. Changes are inherent to life and remarkable are the changes that occur during early development, when a body is, built starting from a single cell.

The blood system starts forming during embryonic development. Here the foundation is laid to ensure a life-long supply of cells to carry oxygen and nutrients and to protect us against pathogens. As we grow up, so do the stem cells of the blood system, changing to accommodate the needs of an adult body.

It has been a fascinating journey - trying to understand the mechanisms that make the fetal to adult transition tick. Learning how developmental timing impacts on the generation of functionally distinct cells making the immune system a chimera of fetal and adult derived protection and following molecular switches like Lin28b in turning on fetal cellular states and B lineage plasticity.
Abstract

B-1a cells are innate-like lymphocytes that develop primarily during fetal and neonatal life, whereas adult bone marrow (BM) hematopoietic stem cells (HSCs) preferentially give rise to follicular B-2 cells. Functioning at the interface of the innate and adaptive immune systems, B-1a cells provide a non-redundant first line of defense prior to the temporally delayed establishment of a B-2 cell response. The underlying causes for the developmental attenuation in B-1a potential remain poorly resolved. HSCs undergo a functional switch in neonatal mice hallmarked by a decrease in self-renewing divisions and entry into quiescence. The timing of this switch around 3 weeks of age correlates with the change in B cell output from B-1a potent to predominantly B-2 restricted.

We hypothesized that the cellular basis for this developmental attenuation in B-1a cell output is a consequence of a shift in stem cell state during ontogeny. Using cellular barcoding for in vivo single-cell resolution analyses, we found that fetal liver definitive HSCs gave rise to both B-1a and B-2 cells. To directly assess whether a developmental shift in HSC state can lead to a selective loss in B-1a potential on a per cell basis, we performed longitudinal comparison of repopulation potential by following barcoded founder cells across serial transplantations. Whereas B-1a potential diminished over time, B-2 output was maintained. B-1a potential could be reinitiated in a subset of adult HSCs by ectopic expression of the RNA binding protein LIN28B, a key regulator of fetal hematopoiesis. This coincided with the clonal reversal to a fetal-like elevated self-renewal and repopulation potential. These results anchor the attenuation of B-1a cell output to fetal HSC behavior and demonstrate that the developmental decline in regenerative potential represents a reversible HSC state.

While these data made clear that developmentally restricted hematopoietic origins cannot fully account for the postnatal decline in B-1a output, the underlying mechanism for the positive selection and output of B-1a cells remains elusive. Recent studies showed that ectopic expression of Lin28b in adult pro-B cells was sufficient to potentiate fetal-like B-1a cell output. This led us to next hypothesize that Lin28b may play an important role during the latter part of B lymphopoiesis to potentiate the positive selection of B-1a cells early in life. We showed that CD5
levels of B-1 cells are developmentally set in the immature B cell stage and correlates with self-reactivity. Genetic perturbation studies show that Lin28b is necessary and sufficient for efficient positive selection of B-1a cells and potentiates neonatal immature B cell CD5 expression in a dose dependent fashion. Importantly, our results uncouple positive selection from specific B cell receptor identities, implicating the heterochronic RNA-binding protein LIN28b as the missing link that regulates the developmental attenuation in B-1a cell output through relaxing the permissiveness of B cell selection. Our findings shed light on the unique ability of B-1a cells to escape tolerance and undergo T cell like positive selection.

Finally, with ongoing investigations of developmental changes in chromatin accessibility between fetal and adult HSCs we have started to dissect the layers in regulation of a fetal HSC state. Interestingly, we find that regulation of the fetal HSC transcriptome relies more on a post-transcriptional layer compared to adult HSCs. This is consistent with the fetal specific expression pattern of the post-transcriptional regulator Lin28b.

Collectively this thesis work has elucidated fetal HSC state and Lin28b associated mechanisms in the attenuation of B-1a cell output during the transition from fetal to adult B lymphopoiesis.
Abbreviations

5FU 5-fluorouracil
AGM aorta-gonad-mesenephros
Arid3A AT-rich interaction domain 3A
B-CLL B cell chronic lymphocytic leukemia
BCR B-cell receptor
BM bone marrow
Btk Bruton’s tyrosine kinase
CLP common lymphoid progenitor
D diversity
DC dendritic cells
E embryonic day
Ebf1 early B cell factor
EHT endothelial to hematopoietic transition
EMP erythro-myeloid progenitor
ER endoplasmatic reticulum
Flt3 fms-like tyrosine kinase 3
FO B follicular B cells
Hmga2 high-mobility group AT-hook 2
HSA heat stable antigen 24
HSC hematopoietic stem cell
HSPC hematopoietic stem and progenitor cells
Igf2 insulin growth factor 2
Igf2bp Igf2 binding protein
IgH immunoglobulin heavy chain
IgM immunoglobulin M
IL7 interleukin 7
iNKT invariant natural killer T
J joining
LMPP lymphoid primed multipotent progenitor
LSK Lineage\(^{\ast}\)Sca-1\(^{\ast}\)cKit\(^{\ast}\)
miRNA microRNA
mTOR mammalian target of rapamycin
MZ marginal zone
nAbs natural antibodies
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>PC</td>
<td>phosphorylcholine</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol-3-kinase</td>
</tr>
<tr>
<td>PtC</td>
<td>phosphatidylcholine</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
</tr>
<tr>
<td>RAG</td>
<td>recombination activating gene</td>
</tr>
<tr>
<td>RNP</td>
<td>ribonucloprotein particle</td>
</tr>
<tr>
<td>RSS</td>
<td>recombination signal sequence</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SDF-1</td>
<td>stromal cell derived factor 1</td>
</tr>
<tr>
<td>SHP-1</td>
<td>Src homology region 2 domain-containing phosphatase 1</td>
</tr>
<tr>
<td>SLAM</td>
<td>signalling lymphocytic activation activation</td>
</tr>
<tr>
<td>TCR</td>
<td>T cell receptor</td>
</tr>
<tr>
<td>TdT</td>
<td>terminal deoxynucleotidyl transferase</td>
</tr>
<tr>
<td>TSLP</td>
<td>thymic stromal lymphopoietin</td>
</tr>
<tr>
<td>V</td>
<td>variable</td>
</tr>
<tr>
<td>YS</td>
<td>yolk sac</td>
</tr>
</tbody>
</table>
The adult B cell compartment – a developmental chimera

The immune system has evolved through millions of years to become a sophisticated defense system that enables our survival by protecting us against pathogens. The vertebrate immune system consists of two temporally distinct arms of response to infection; the rapid innate immune response acts as the first line of defense during early phases of infection through recognition of broadly conserved molecular patterns, while the adaptive immune response acts at later stages after clonal expansion of antigen specific effector cells. Traditionally the cellular constituents of innate immunity are myeloid blood cells while adaptive immunity relies on a somatically diversified repertoire of antigen receptors of T and B lymphocytes [1, 2].

A group of innate-like B (marginal zone and B-1 B cells) and T lymphocytes (γδT and invariant natural killer T cells (iNKT)) function at the interface of innate and adaptive immunity, with characteristic properties from both arms. These cells are often tissue-resident and harbor semi-invariant self-reactive receptors enabling the recognition of qualitatively distinct antigens from their non-innate lymphocyte counterparts. Interestingly these innate-like lymphocytes are often of early life origin where they initially dominate in numbers. They continuously shape the adult immune system by sustaining their presence throughout the life of an organism [3-5].

In the layered immune system hypothesis proposed by the Herzenberg laboratory (Stanford University) in 1989, they suggested that the progressive evolution of the immune system is “recorded” in the populations of innate-like and adaptive lymphocytes [6]. This concept of an evolutionarily layered immune system in the mouse was based on seminal transplantation studies, showing that the first B cells to emerge during ontogeny was an innate-like subset of B cells (B-1), with distinct and primitive functions compared to B cells that appear, later in development (B-2). B-1 cells were proposed to represent an early evolutionary layer in the immune system [7, 8]. The distinct complementary functions of innate-like lymphocytes
argue for their conservation as an important part of the evolved immune system rather than a vestigial appendix.

Early life derived B-1 cells

The B cell compartment exemplifies the concept of the adult immune system as a developmental chimera (Figure 1). In the adult B cell compartment, fetal derived innate-like B-1 cells combine with adult derived B-2 cells to provide non-redundant roles in protection. Unlike their follicular B (FO B) counterparts, which are continuously replenished from BM progenitors, mature B-1 cells are long-lived and can self-renew in the periphery [9, 10]. This means that despite the predominant generation of B-1 cells early in ontogeny, the mature population is self-sustaining throughout the life of the animal.

Figure 1
The adult B cell compartment – a developmental chimera. In the adult B cell compartment fetal derived B-1 cells combine with adult generated B-2 cells to provide non-redundant immune functions and optimal protection for the host.

The identification of B-1 cells was a serendipitous discovery as a result of progress in the development of flow cytometry at Stanford University and the identification of the CD5 surface marker on human B cell chronic lymphocytic leukemia (B-CLL). CD5 had previously been exclusively associated with expression on T cells [11]. B-CLL is a disease of the elderly and thus it was surprising that the CD5⁺ B-1 cells identified in normal mice were found to develop in early ontogeny and with the highest abundance during early life [12]. Although CD5 is expressed more promiscuously on human B cell subsets, the CD5⁺ compartment is also more abundant in human neonates compared to adults [13, 14].

B-1 cells are largely tissue-resident, contributing to the majority of B cells found in the peritoneal and pleural cavities (30-70%) as well as a smaller fraction of the spleen and BM cells in mice [15]. B-1 cells are immunophenotypically defined as
CD19+ B220\textsuperscript{low/-}CD43\textsuperscript{+}CD23\textsuperscript{+}IgM\textsuperscript{high}IgD\textsuperscript{low}CD11b\textsuperscript{+/int} in the peritoneal cavity and CD19+ B220\textsuperscript{low/-}CD43\textsuperscript{+}CD23\textsuperscript{+}IgM\textsuperscript{high}IgD\textsuperscript{low} in the spleen [16, 17]. Two subsets of B-1 cells are distinguished based on their CD5 expression; CD5 positive (B-1a) and CD5 negative (B-1b) [18]. While initially proposed to be closely related in terms of both function and development, later studies showed division of labor between the subsets in response to infection [19]. In addition, studies on the developmental timing of B-1a and B-1b generation have shown clear differences. Lineage tracing across 36 weeks in adult (8-10 weeks old) mice show low influx (<5%) from adult BM HSCs into the B-1a subpopulation [20] while about 50% of B-1b cells can be regenerated from transplantation of adult BM [9].

An important difference between B-1 and B-2 cells is the secretion of broadly self-reactive immunoglobulin M (IgM) natural antibodies (nAbs), which provide tissue homeostatic function through clearance of apoptotic cells [21] and protection from bacterial toxins [22]. The best characterized nAbs recognize phosphorylcholine (PC), a group of phospholipids found in the cell membranes of pathogenic bacteria and self-membranes [23]. B-1 cells are the main source of nAbs, they are characterized by a recurrent repertoire with few N-nucleotide additions and a restricted V\textsubscript{H} usage [24], enriching for self-reactive B cell receptors (BCRs). The most abundant reactivity in the B-1 population (5-10%) is against phosphatidylcholine (PtC), a PC moiety displayed by senescent erythrocytes [25]. Another well-studied example of unique B-1 contribution to PC nAbs is the exclusive generation of T15 idiotype antibodies from CD5\textsuperscript{+} B-1 cells [26]. The requirement for this specificity is exemplified by its protective function during Streptococcus pneumoniae infection [27]. The loss of this protection upon enforced expression of terminal deoxynucleotidyl transferase (TdT) during early development leads to higher mortality upon infection [28]. Unlike their B-2 counterparts B-1 cells can spontaneously secrete nAbs without prior stimulation [29]. Upon stimulation with particulate bacterial antigen B-1 together with marginal zone (MZ) B cells differentiate into IgM secreting plasma cells in a T-independent manner to clear infection [30]. Interestingly, self-reactive specificities of IgM from the cord blood of human neonates is highly correlated between newborns, but not with maternal blood or milk [31, 32]. This suggests a parallel between human neonatal B cells and murine early life B-1 cells; both displaying spontaneous secretion of natural IgM with a restricted repertoire biased towards self-reactive specificities shared between individuals.
Table 1
Key differences between B-1 and B-2 cells

<table>
<thead>
<tr>
<th></th>
<th>B-1 cells</th>
<th>B-2 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontogeny</td>
<td>Predominantly fetal and neonatal</td>
<td>Fetal, neonatal and adult</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Self-renewal in the periphery</td>
<td>Continuously replenished from BM HSCs</td>
</tr>
<tr>
<td>Main locations</td>
<td>Body cavities and spleen</td>
<td>Spleen and lymph nodes</td>
</tr>
<tr>
<td>Repertoire</td>
<td>Semi-invariant, self-reactivity</td>
<td>Diverse</td>
</tr>
<tr>
<td>Function</td>
<td>Rapid, first line of defense</td>
<td>Delayed, specific response</td>
</tr>
</tbody>
</table>

B cell development from fetus and adult

B cell development is an ordered process of distinct stages with gene rearrangements aimed at the final generation of a functional BCR. These rearrangements of the immunoglobulin gene segments provide mature B cells with a highly diverse set of possible receptor configurations enabling the recognition of a large variety of pathogens. This process is orchestrated by regulatory networks of transcription factors for priming, specification and commitment to B cell differentiation and is accompanied by distinct changes in cell-surface immunophenotype.

The BCR consists of an immunoglobulin heavy chain (IgH) encoded by gene loci containing variable (V), diversity (D) and joining (J) gene segments and a light chain (Igκ or Igλ) encoded by V and J gene segments. The lymphoid specific recombination activating genes Rag1 and Rag2 induce double stranded breaks between flanking recombination signal sequences (RSSs) and the coding V,D and J gene segments [33, 34]. Before ligation of the gene segments the enzyme terminal deoxynucleotidyl transferase (TdT) adds random nucleotides to the junctions (N-additions), further increasing the diversity of final receptor specificities [35, 36].

Quality control of this somatic VDJ recombination is regulated by checkpoints at the pre-B stage and the immature B cell stage in early B cell development [37].

B-2 cell development in the adult BM

In adult hematopoiesis, B cells are continuously replenished from progenitors in the BM. Lymphoid restriction is first found in the common lymphoid progenitor (CLP) population, with the potential to generate B, T, natural killer (NK) cells and a subset of dendritic cells (DCs) [38], while lacking erythroid and myeloid potential. This population was initially defined by the surface markers Lin⁻c-Kit⁺Sca-
The CLP population has later been shown to possess considerable heterogeneity. Ly6D$^+$ expression on CLPs defines a subset retaining potential for adaptive T and B lymphocytes but lacking NK potential [41]. Within the Ly6D$^+$ CLPs high expression of Rag1 and $\lambda_5$ marks B committed progenitors [42]. The IgH starts to recombine $D_h$-$J_h$ gene segments when cells reach the CLP stage [43]. The progression from Ly6D$^+$ CLP to the pro-B cell stage requires the transcription factor early B cell factor 1 (Ebf1). This was demonstrated in Ebf1 deficient mice where B cell development is blocked at a B220$^+$CD43$^+$ stage without $D_h$-$J_h$ gene rearrangements [44]. FOXO1 transcription factor is required for transcription of Rag1 [40] and was shown to act in a positive feedback circuit with Ebf1 to enable early B cell developmental stages [45].

At the pro-B cell stage (Hardy fraction B/C) both RAG proteins and TdT are highly expressed and the heavy chain is recombining $V_h$-$DJ_h$ gene segments. In the pursuing early pre-B stage (fraction C') the heavy chain is fully recombined ($V_h$-$DJ_h$) and coupled to $\mu$-constant region ($\mu$-heavy chain). The $\mu$-heavy chain is tested in the pre-BCR checkpoint [46]. The pre-BCR complex consists of the $\mu$-heavy chain binding to the surrogate light chain (SLC) composed of $\lambda_5$ and VpreB, together with CD79a/b [34]. Upon successful signalling from the pre-B cell receptor rapid proliferation starts simultaneously with sharp downregulation of RAG proteins and TdT [47, 48]. As early pre-B cells exit cell cycle they become small and enter the late pre-B stage (fraction D). Expression of RAG proteins and TdT goes up and the B cell finishes rearrangement of the light chain locus $V_L$-$J_L$ [49-51]. At this stage, the B cell has the full structure of the BCR and the second checkpoint steps into action (Figure 2). The final outcome of this checkpoint based on the signalling strength of the receptor is either 1) positive selection into the immature B cell pool for further maturation and migration to the spleen or 2) deletion by apoptosis. However, B cells that are initially negatively selected have a second chance to generate a BCR with the right signalling through receptor editing [37].
Fetal B cell development – clues to the origins of qualitatively distinct B cell subsets in the adult B cell compartment

While fetal B cell development progresses through similar stages as identified in adult B cell development [50], several key differences could affect the composition of mature B cell output. During fetal development, myeloid potential follows B-potent progenitors longer than in the adult. The immunophenotypic E14.5 fetal counterpart of the adult CLP Lin⁻c-KitintSca-1intIL7R⁺Flt3⁺ population retain myeloid macrophage potential [52]. More committed B progenitors in FL were found to be bi-potential for B cells and macrophages, further highlighting the link between B and myeloid fates in the fetus [53]. A subset of the first CD19⁺ pro-B cells found in FL E13.5 are CSF1R⁺ and still retain myeloid potential while this population is absent in the adult pro-B population [54]. Additionally, counter to adult CLPs Ly6D is not detected until after E15 in fetal development, and B cell priming in the fetal CLP compartment is instead marked by heat stable antigen 24 (HSA) high surface expression [55]. While adult B cell developmental stages are located within the BM, fetal B cell progenitors are spread across different anatomical locations. In addition to FL a small amount of CD19⁺B220⁺CD43⁺ pro-B can be found in the fetal spleen, at E13.5 [56]. From the pro-B cell stage cells progress to μ-chain expressing pre-B in the FL, BM and spleen with the first IgM⁺ B cells maturing around E17 [57, 58].

Examples of differences in regulation of B cell development between FL and adult BM are observed in the interleukin 7 (IL7) receptor and the PU.1 transcription factor deficient mice. While adult B cell development is arrested prior to CD19 expression in IL7 receptor deficient mice, fetal pro-B cells are generated from both E15 and
neonatal mice although less efficiently than in wildtype. The decrease in mature B
cells of IL7 receptor deficient mice is more severe in the B-2 cell compartment
compared to the B-1 population [59-61]. This could be connected to the
responsiveness of fetal but not adult pro-B cells to thymic stromal lymphopoietin
(TSLP) [60] and Flt3 signalling compensating for IL7 deficiency during fetal
development [62]. PU.1 deficiency abrogates B-2 development but is not a
prerequisite for B-1 cell development [63]. FL B-1 restricted progenitors are higher
in frequency during ontogeny compared with adult BM [64] and are differentially
affected by PU.1 dosage at different times during ontogeny. While CD19+ cells were
completely absent in PU.1 deficient FL at E16.5, they appear at E18.5 and
preferentially generate B-1b cells [65].

These examples highlight that multiple differences exist between fetal and adult B
cell development in immunophenotype, lineage plasticity, cytokine and
transcription factor requirements.

**HSC-independent B-1 progenitors from fetal development**

Recent reports have suggested an HSC-independent origin for the B-1a population,
by fetal progenitors without B-2 potential [66]. The earliest B-potent progenitors
develop independently of hematopoietic stem cells before their emergence at E10.5
from the aorta-gonad-mesonephros (AGM) region [67]. While early progenitors of
B-1a restricted potential were first identified from embryonic para-aortic
splanchnopleura (Psp) at E8.5 [68], multi-lineage potential prior to circulation was
later shown to arise from the same region demonstrating that the site contains
progenitors of the hematopoietic stem and progenitor (HSPC) compartment [69]. B-
1 and MZ B restricted progenitors that lack FO B-2 potential were later proposed to
arise in the E9 yolk sac (YS), as demonstrated by transplantation of circulation
deficient donors. However, only the B cell lineage was analysed in this study, and
thus it is possible that these progenitors have the potential to generate cell types
other than B-1 and MZ [70]. In fact, it was demonstrated that immune-restricted
progenitors with combined B, T and myeloid potential and transcriptional priming
at the single cell level emerge in the YS at E9.5 [71]. The E13 fetal omentum is
another site for B-1 potent progenitors, but it has not been resolved whether these
progenitors emerge in situ or migrate from the FL [72]. A recent study suggested
that FL HSCs are unable to generate B-1a cells, and in extension proposed a model
attributing all B-1a contribution in the mouse to the HSC-independent emergence
of B-1 restricted progenitors [66, 73]. While it is clear that B-potent progenitors are
generated prior to HSC emergence, the contribution of these cells to the adult B cell
pool during unperturbed hematopoiesis remains to be explored. (Paper I and general discussion).

*Developmental factors contribute to generation of a restricted BCR repertoire early in life*

Some of the differences in repertoire between B-1 and B-2 cells can be explained by the predominantly fetal development of B-1 cells. One factor is the low expression of TdT in fetal progenitors (Hardy laboratory Immgen) leading to a lack of N-additions in BCRs of fetal and neonatal B cells. Consequently, the BCR repertoire of fetal derived B cells has lower diversity compared to adult BM derived B cells where TdT is expressed [74, 75]. The B-1 and MZ B cell populations both have low levels of N-additions, suggesting that these B cell subset are largely derived from fetal development [76]. The B-1 repertoire changes with age and a higher percent of sequences with N-nucleotide additions appears suggesting input to the B-1 population from the BM after expression of TdT initiates [77]. Lack of TdT however, is not sufficient to explain the restricted $V_H$ usage in the B-1 compartment enriched for $V_H^{11}$ and $V_H^{81x}$, associated with self-reactive natural antibodies [78]. An explanation for this differences in repertoire is positive selection by binding to self-antigen, which has been clearly demonstrated to be a prerequisite for B-1 cells [79]. However, the effect of this mechanism on maintenance versus development remains unclear. Alternative explanations have focused on a difference in the pre-BCR checkpoint between fetal and adult. The self-reactive $V_H$ segments incorporated during fetal B cell development are actively suppressed in the adult [80-82]. The inefficient pairing of B-1 biased $V_H^{11}$ and $V_H^{81x}$ with the SLC indicate a more lenient quality control in the pre-B receptor checkpoint during fetal development [83-85]. This concept is supported by studies of $\lambda 5$ deficient mice which lack B-2 B cells while still generating B-1 cells [86] and SLC deficient mice harboring increased levels of self-reactive antibodies [87]. However, a mechanism for how FL B cell development could enable a more lenient selection threshold remains to be elucidated (Paper II).

**What makes a B-1? - Lineage model versus Selection model**

Shortly after the discovery of B-1 cells controversy divided the field regarding the point of divergence between B-1 and B-2 fate choice. Centred around two main schools of thought the discussion on B-1 commitment is still running now more than
30 years after [66, 88-90]. On one side the lineage model, inspired by the layered immune system hypothesis [6], proposes a division of B-1 and B-2 cells into separate developmental lineages with distinct progenitors from different developmental times. In this model, the point of divergence between B-1 and B-2 is prior to expression of surface BCR. Oppositely, the selection model in simple terms states that commitment into the B-1 population is dependent on instructive BCR signalling. The selection model links the B-1 phenotype to the repertoire found in this population.

Early work suggested that B-1 cell generation is exclusive to fetal hematopoiesis as transplantation of BM generated low amounts of B-1 cells [7, 8]. Transfers of single BM HSCs likewise were concluded to lack B-1a potential [91]. The restricted generation of B-1 cells during a limited fetal time window was proposed to favour the lineage model, with fetal origin implicating distinct B-1 progenitors. For a while this notion remained a subject of discussion, but several studies have now shown that adult BM generates B-1 albeit with lower efficiency than fetal and neonatal hematopoiesis. Using a conditional Rag1 model, B-1 cells were generated from adult BM when Rag1 was induced and B cell development turned on [92]. Furthermore, during unperturbed hematopoiesis adult BM HSCs contribute with <5% to the B-1a population [20] and around 50% to the B-1b population (Säwen et al. in revision). Finally, the best demonstration of B-1 influx from adult BM is the presence and increase in N-addition containing sequences with age, which can only be generated with TdT expression after birth [75, 77].

The identification of a B-1 specified progenitor without the potential to generate B-2 cells is a key piece of evidence in support of the lineage model [64, 93]. This progenitor population defined as lineage^{AA4.1^{-}B220^{-}CD19^{+}} has B and myeloid potential in vitro [94], but a B-1 specific potential in vivo. This finding was extended with a clonal in vitro study on CLPs which showed a commitment to either B-1 or B-2 but no bi-potentiality at this developmental stage [95]. The spleen transitional B-1a committed splenic progenitor dependent on IκBNS is in line with both models as the B-1 restriction happens after the expression of a functional BCR [96].

HSC-independently generated B-1a committed progenitors have not been prospectively isolated and therefore remain to be thoroughly characterised. An origin completely outside the HSC system would however be strongly in favour of the lineage model. The recent study suggesting that B-1 cells are exclusively derived from fetal progenitors independent of HSCs further builds evidence for this extreme lineage model [73]. (Paper I)
The first studies inspiring the selection model identified induced differentiation to the B-1 phenotype upon crosslinking of the BCR and in vitro culturing [97]. A receptor-dependent change of phenotype has since been presented more elegantly by in vivo switching from a B-2 BCR to a PtC B-1-typical BCR which induces both phenotype, and localization, as well as survival and response properties characteristic of B-1 cells [98].

Selection (or maintenance) of B-1 cells based on recognition of self-antigen has been clearly demonstrated [79] and used as an argument for the selection model. In line with this, the repertoire of B-1a cells from both peritoneal cavity and spleen is more restricted, self-reactive and repetitive compared to the highly diverse repertoire of follicular B-2 cells [99]. In T cells CD5 expression correlates with TCR signaling strength [100] and is linked to self-reactivity of the receptor [101]. CD5 is highly expressed on early life generated T cells and has been linked to negative regulation of BCR signaling in B-1 cells [102, 103].

Several studies implicate signalling strength downstream of the BCR in the fate choice decision between B-1 and B-2. Increased signalling strength of the BCR through higher density of the receptor showed a differentiation bias towards B-1 cells [104]. In accordance with this, deletion of proximal BCR associated signal-attenuating proteins as the phosphatases SHP-1 and PTEN or the inhibitory receptor Siglec-g promotes B-1a representation over B-2 in the mature B cell population [105-107]. Moreover, deletion of positive regulators of proximal BCR signalling as CD19, Btk and PI3K decreases the B-1 fraction of the B cell population [108-110]. The B-1 cell population correlates in a dose-dependent manner with the level of positive-enforcers of BCR signalling, such as CD19 [111]. These studies demonstrate the importance of signalling strength, but fail to distinguish between the effects on development versus maintenance.

As explanations for the B-1 versus B-2 fate decision, the lineage model and the selection model are non-mutually exclusive, but clearly there are missing links that could explain the irreconcilable conclusions drawn on either side. The strongest argument in favour of the lineage model is the B-1 restricted progenitor prior to VDJ rearrangement. On the other side, the impact of signalling strength for the B-1 versus B-2 commitment is unquestionable. A missing perspective is the impact of developmentally regulated factors governing B cell development.
Lin28 a fetal ON switch

Lin28 was first identified in Caenorhabditis elegans in 1984 as part of the heterochronic family of genes [112]. Gain and loss of function mutants displayed delayed or advanced developmental maturation respectively demonstrating the role of Lin28 as a switch for stage specific programs during larval development [113]. It soon became apparent that the Lin28 switch is not unique to the worm model organism. The specific expression pattern of Lin28 during early development is conserved across evolution from Caenorhabditis elegans [113], Drosophila [114], zebrafish [115], Xenopus [116], mouse and human [114]. Lin28 is highly expressed in murine embryogenesis across tissues and embryonic layers until E9.5 after which Lin28 expression becomes more tissue-specific [117].

The two mammalian homologs of Lin28, Lin28a and Lin28b (collectively referred to as Lin28) share common RNA-binding domains (a cold-shock domain and two CCHC-type zinc-finger domains), which are the structures required for their interaction with primary and precursor miRNAs of the Let-7 family [118]. The expression of Lin28 and Let-7 are inversely correlated, with expression of Lin28 highest during ontogeny and Let-7 peaking in the adult. Together they form an axis for the transition from fetal to adult developmental stages [119].

Lin28 expression has been tightly linked to “stemness”. Early on it was found to be highly expressed in embryonic stem cells and downregulated during differentiation [114, 120]. This link to was further corroborated by the demonstration that Lin28a together with Nanog, Oct4 and Sox2 could reprogram human fibroblasts to pluripotent stem cells [121]. An important role of Lin28 during reprogramming is regulation of metabolism [122], which together with its pro-growth phenotype constitute the main characteristics of Lin28 as a proto-oncogene [123]. Interestingly, this promotion of stemness in Lin28a overexpressing mice allows for enhanced tissue regeneration in epidermal hair growth, digit regrowth and pineal tissue repair through an increase in the cellular bioenergetic state [124].
Lin28 a multifaceted post-transcriptional regulator

The best known mechanism for Lin28 function is the negative regulation of Let-7 biogenesis. Let-7 belongs to the micro RNA (miRNA) family (~22nt length) of small RNAs which are known for their role in mRNA destabilization and translational repression. After transcription, primary miRNAs require further processing to become active in their mature form [125]. Lin28 has been shown to regulate this processing at two different steps. First by interfering with the microprocessor complex (Drosha and DGCR8) binding to primary Let-7s during nuclear processing [126]. Secondly, Lin28a further prevents cytoplasmic processing by recruiting the terminal uridylyl transferase TUT4 and mediating uridylation of precursor Let-7s. This modification prevents Dicer processing and leads to precursor Let-7 degradation [127, 128].

The Let-7 family of miRNAs is highly evolutionarily conserved and displays a heterochronic expression pattern during ontogeny, opposite to that of Lin28 [129, 130]. Among Let-7 targets that are de-repressed by Lin28 expression are oncogenes and proliferation associated mRNAs such as c-Myc, n-Myc, Lin28 itself [131], Ras [132], Hmga2 [133], cyclin A2 and Cdc34 [134]. These targets clearly show the repression of proliferation by Let-7, its role as a tumour suppressor and by extension the role of Lin28 as a proto-oncogene.

In addition to Let-7 dependent mechanisms, Lin28 functions in post-transcriptional gene regulation by directly binding to mRNA. The first mRNA shown to directly bind Lin28a was insulin growth factor 2 (Igf2) in differentiating myoblasts. Lin28a and Igf2 mRNA were found in RNase-sensitive ribonucleoprotein particles (RNPs) together with ribosomal proteins and Igf2-binding proteins (Igf2bps). These complexes associated with polysomes, leading to increased translation of Igf2 [135]. In addition, Lin28 was shown to induce proliferation by directly binding to the mRNAs of cell cycle regulators (cyclin A, cyclin B and CDK4) resulting in an increase in their protein abundance [136, 137]. Subsequently, multiple studies have investigated the RNA-interactome of Lin28 in different cell types. A study from 2012 reported repression of Lin28a bound mRNAs in embryonic stem cells [138]. However, multiple other studies from the same period all recorded increased translation of Lin28 bound mRNAs [137, 139-141]. These studies showed that Lin28 bound transcripts were enriched for mRNA encoding RNA-binding proteins, with many splicing factors identified within this group. In line with this Lin28a expression in somatic cells lead to increased alternative splicing patterns [139]. Another group of RNA-binding protein transcripts bound by Lin28 encode for
ribosomal proteins and translation factors, suggesting that Lin28 heightens the cellular bioenergetic state through increased ribosome biogenesis [137, 139-141]. These conclusions are strengthened by the impact of Lin28a on ribosomal RNA processing and nucleolar numbers [142].

A less explored mechanism of Lin28 is coupled to the long non-coding RNA H19. H19 is expressed from the maternal allele of the imprinted Igf2-H19 locus during embryogenesis [143]. Lin28 and H19 expression patterns and pathways integrate at multiple points. Both are highly expressed during fetal development, and they exhibit a muscle tissue restricted expression pattern in adult life, with decreased expression of either Lin28 or H19 leading to impaired glucose homeostasis [144, 145]. Additionally both Lin28 and H19 are part of the onco-fetal program overexpressed in certain cancers [146] and form complexes with Igf2bps, leading to pro-growth phenotypes [147]. Like Lin28 H19 binds to Let-7 but the function for this interaction remains subject to discussion [144, 148, 149]. Ectopic expression of Lin28b increases the abundance of H19 (unpublished data and [150]) and recently overexpression of H19 has been reported to increase Lin28a expression [151]. While there seem to be a connection between the regulation and function of Lin28 and H19, the mechanism and functional outcome of this partnership are yet to be investigated.

**Lin28 impact on growth and metabolism**

Lin28b is a genetic determinant for the timing of puberty in human transition from childhood to adulthood [152]. Several loci with genes bearing let-7 target sequences are associated with increased height - among these is Lin28b [153]. Consistent with this, a Lin28a overexpressing mouse model shows increased body size and delayed timing of puberty compared to littermate controls [154]. Furthermore, deficiency in Lin28a leads to delayed onset of puberty in mice and causes dwarfism starting from E13.5 with a 30-50% decrease in adult body size [145, 155]. Thus, Lin28 is a conserved regulator of mammalian organismal growth.

Clues to how Lin28 may control body size come from Igf2; a main determinant of intrauterine and perinatal growth through the Igf2/ insulin-PI3K-mTOR –pathway [156]. Lin28 directly upregulates several of the key players in this pathway. As discussed earlier, Igf2 protein translation itself is increased by Lin28a [135]. Furthermore, overexpression of Lin28 in adult mice increases glucose tolerance and resistance to obesity and diabetes through increased expression of Igf1R, InsR, Irs2,
PIK3IP1, Akt2, Tsc1 and Rictor (constituents of the Igf2/insulin-PI3K-mTOR pathway). Furthermore, increased phosphorylation of S6, Akt and 4Ebp1 implicate Lin28 in the activation of mTOR [157]. Lin28 induced modulation of glucose metabolism appears to be mediated through both Let-7 dependent and independent mechanisms. While many of the Igf2/insulin-PI3K-mTOR pathway constituents contain Let-7 seed sequences, direct Lin28a-binding of mRNA coding for these signalling factors has likewise been shown [141]. The influence of Lin28 on glucose metabolism was further corroborated by studies of Lin28 deficient mice, resulting in impaired glucose uptake and decreased glycolytic intermediates [145].

In an exciting study from 2013, the remarkable regenerative potential of Lin28 induction in tissue repair was demonstrated in the context of digit and hair regeneration [124]. This study extended the previous understanding of Lin28a as a metabolic regulator by showing its ability to increase both the oxidative phosphorylation and glycolysis pathways. In support of this, Lin28a was found to bind to the mRNAs of multiple rate limiting enzymes in both these pathways (Pfkp, Pdha1, Ndufb3/8).

Collectively these studies show an evolutionarily conserved effect of Lin28 on body size and tissue regeneration through the Igf2/insulin-PI3K-mTOR pathway, resulting in an increased cellular bioenergetic state (Figure 3).

![Figure 3](image)

Summary of Lin28 targets through direct binding to mRNA and repression of Let-7 miRNA.
Lin28 in pediatric cancer

Embryonic development and transformed cancer cells share several key requirements including proliferation, self-renewal and the ability to migrate. The onco-fetal Lin28 proteins are associated with different human malignancies, where they promote transformation by retaining a stem-like undifferentiated progenitor state [158]. Interestingly, 25% of reported Lin28 overexpressing cancers are pediatric (0-15 years), a high frequency considering pediatric cancers constitute less than 1% of all newly diagnosed cancers [159].

An example of Lin28b overexpression in pediatric cancer is neuroblastoma where Lin28b overexpression is associated with poor survival. Lin28b knock down triggered growth arrest and differentiation marker upregulation in a human neuroblastoma cell line. Importantly, mice with constitutive overexpression of Lin28b in the neural crest developed tumors 30-50 days after birth in primary sites of human neuroblastoma and displayed typical markers for the disease. This demonstrated the tumor initiating potential of Lin28b in neuroblastoma [160]. More recently, hepatocyte specific Lin28b overexpression was shown to be sufficient to drive liver tumorigenesis in vivo. Expression of α-fetoprotein and Igf2 indicated a fetal state of the tumour cells [161]. Lin28b overexpression has also been associated with childhood cancer in the blood system. Lin28b defines a subset of juvenile myelomonocytic leukemia cases correlating with poor prognosis and fetal hemoglobin expression. This Lin28b expressing subgroup accounts for around 50% of juvenile myelomonocytic cases [162].
A fetal hematopoietic stem cell state

Hematopoiesis, the making of blood, relies on the hematopoietic stem cell for continuous replenishment of mature blood lineages of both myeloid and lymphoid branches. The HSC compartment sustains life-long hematopoiesis, through differentiation of progressively more lineage-restricted progenitors specifying mature cells in continuous trajectories orchestrated by intricate circuits of transcription factors and epigenetic modifications [163]. During unperturbed hematopoiesis, immediate regeneration is handled by progenitors just downstream of the HSC undergoing active proliferation and differentiation [164]. Over time, however, HSCs contribute to most mature cell lineages with the exception of a few long-lived immune cell subsets [20]. The HSC compartment is largely quiescent and serves as a backup reservoir which is activated upon external stimuli [165, 166]. Traditionally, the HSC has been functionally defined by its multi lineage potential at the single cell level in vitro and by its capacity for self-renewal assessed by serial transplantation in vivo [167].

During fetal development the blood system is undergoing extensive dynamic changes over a short time span. Counter to the adult system, which resides in a homeostatic state fetal hematopoiesis has a different agenda – to build a complete blood system from scratch. A faster progression through the hematopoietic system for FL HSCs compared to adult was elegantly demonstrated by a Tie2 lineage tracing mouse model. While it took months for adult HSCs to contribute significantly to the peripheral blood system, the progenitor output of FL HSCs marked at E10.5 approached equilibrium already 1 week after birth [168]. This demonstrates the fast kinetics of fetal HSCs primed to generate output.

In the murine blood system, a transition from a fetal to an adult HSC state takes place at around 3 weeks of age, marking a reduction in proliferation, self-renewal and repopulation capacity [169]. This switch coincides with a qualitative change in lymphocyte output and a transition to stringent IL7 receptor dependence in B lymphopoiesis [170, 171]. This chapter is aimed at highlighting differences between the fetal and adult HSC state.
Comparison of fetal liver and adult HSCs

Cycling and biosynthesis

The BM HSC compartment relies on self-renewal to sustain its population size as there is no new HSC input after fetal development [172]. Homeostasis of the population requires the balancing of differentiation responses to external challenges with symmetric self-renewing divisions on one hand and death/egress from the BM on the other. This balancing act requires a strictly regulated maintenance of the adult HSC population to sustain and preserve genome integrity during the lifetime of an organism. Adult BM HSCs safeguard long-term maintenance by upholding a quiescent state with low biosynthesis. This is in contrast to fetal HSCs which differ in multiple cellular aspects. While the majority of adult BM HSCs are in the G0 stage of cell cycle (~70%) [165] most FL HSCs are actively cycling more akin to non-self-renewing progenitors in the adult hematopoietic system [173]. In line with this, adult HSCs also have the lowest protein synthesis rate of all HSPCs [174] while FL HSCs in contrast exhibit a high protein synthesis rate [175]. Interestingly, the higher protein synthesis rate in FL translates into a less complex proteome compared to adult HSPCs [176]. A restricted proliferative history in adult BM HSCs correlates with higher HSC function, while active cell cycle status is linked to poor self-renewal and reconstitution capacity after transplantation [165, 166, 177]. In contrast, FL HSCs outcompete their adult counter parts in repopulation capacity and self-renewal while simultaneously displaying highly active cycling behaviour [178]. Oxidative phosphorylation generates ATP for energy consumption but also leads to increased reactive oxygen species (ROS). In adult BM HSCs, high ROS levels are associated with differentiation, exhaustion and loss of self-renewal [179, 180]. Unlike their downstream progenitors, adult BM HSCs favour anaerobic glycolysis while actively suppressing oxidative phosphorylation [181]. This is in contrast to the fetal HSCs which, compared to adult HSCs have enriched expression of oxidative phosphorylation associated genes, increased mitochondrial mass and oxygen consumption and higher ROS levels [182]. The above-mentioned differences highlight an interesting notion, namely that the FL HSC state shares more similarities with non-self renewing multipotent hematopoietic progenitors rather than HSCs in the adult, with respect to cellular and metabolic signatures [183, 184].

The underlying mechanisms allowing fetal HSCs to retain high functional capacity in parallel with high proliferation and oxidative stress are poorly understood. A
recent report provide compelling evidence implicating a unique role for the FL niche in supporting HSC biomass accumulation and expansion. In this study, the high protein synthesis in FL HSCs was found to be accommodated by the secretion of bile acids that alleviate endoplasmatic reticulum (ER) stress. Bile acid deficiency significantly decreases FL HSC numbers demonstrating the essential role of the liver niche for expansion of HSCs [175]. Importantly, this study highlights the existence of fetal specific mechanisms for stress alleviation in the HSC compartment. (Paper III and general discussion).

Differences in surface markers between E14.5 and adult HSCs

Studies on HSCs have been facilitated by their prospective isolation and characterization based on surface markers in combination with assessment of lineage potential and the capacity to reconstitute lethally irradiated recipients. Both in the adult BM and in the E14.5 FL, HSCs reside within the Lineage− Sca-1+cKit+ (LSK) compartment [185, 186]. The identification of HSCs within the LSK compartment by the CD48−CD150+ signaling lymphocytic activation molecule (SLAM) markers is also constant between E14.5 and adult [187, 188]. However while HSCs in the adult BM are negative for the myeloid lineage marker CD11b, most FL HSCs are CD11b+ [173]. This could reflect their state of activation as following 5-fluorouracil (5FU) treatment HSCs have been found to increase their expression of CD11b, coinciding with a rapid recruitment into cell cycle [189]. In addition, within the adult LSK compartment FLT3 upregulation is accompanied by loss of self-renewal and erythroid lineage potential [190, 191]. The same seems to hold true for the majority of HSCs at E14.5 [192], although a recent study reported a rare transient Flt3+ HSC population in the FL [193]. Finally, CD34 expression decreases in the LSK compartment with age, with FL but not adult HSCs positive for this marker [194]. These differences in surface marker expression can represent a change in activation state and different environments between FL and adult BM. Furthermore, dynamic changes in HSC surface markers during early ontogeny can pose a challenge in comparisons with over-reliance on immunophenotype over function.

Transcription factor dependencies

Key differences between FL and adult BM HSCs have been identified in transcriptional regulation. Transcription factors highly expressed in fetal HSCs are often associated with proliferation, while those upregulated after the switch to an
adult state maintain quiescence. Presented here is a non-exhaustive group of developmentally regulated factors that are differentially required by fetal and adult HSCs. Sox17 is a key factor for fetal and neonatal hematopoiesis where it is required for the emergence of HSCs from the hemogenic endothelium [195]. Sox17 is expressed in all E14.5 FL HSCs but expression gradually decreases and is gone in BM HSCs at 8 weeks of age. The timing of Sox17 expression follows the switch from a fetal to adult HSC state at around 3 weeks of age [169]. Importantly induced deletion of Sox17 in neonatal mice (2-5 days old) leads to hematopoietic failure and death, while deletion in 6 weeks old mice showed no effect on hematopoiesis. The requirement for Sox17 at the fetal HSC state was further corroborated by transplantations of Sox17 deficient FL and neonatal BM, both of which showed impaired reconstitution capacity. This highlights the importance of Sox17 for the fetal HSC state and shows a clear difference in developmental requirements [196]. Furthermore, ectopic expression of Sox17 in the adult BM induces fetal-like HSC repopulation potential and phenotype [197]. Another transcription factor whose expression correlates with the developmental switch in HSC state is C/EBPa. However in contrast to Sox17, C/EBPa expression is low in FL HSCs but goes up after birth with an 6-7 fold increase from 2 weeks to 8 weeks of age. Loss of C/EBPa in adult HSCs acts as switch to turn on fetal-like cycling and self-renewal, along with a transcriptional program characteristic of FL HSCs. Thus, C/EBPa expression appears to be involved in the maintenance of quiescence in adult HSCs by repression of fetal proliferation [198]. Similarly, Gfi-1 is also a key transcription factor acting as a “brake” for adult HSC proliferation. In Gfi-1^-/- mice the expression of cell cycle checkpoint regulator p21 is downregulated and the repopulation capacity of HSCs is compromised. However this only happens after 5 weeks of age, indicating that the FL HSC state does not require Gfi-1 [199]. Finally, members of the polycomb family of transcriptional repressors are required for adult HSCs, but their deficiency has little effect on FL HSCs. Both Bmi1 and Eed knockout models show developmentally timed effects with normal numbers of FL HSCs but loss of HSCs after birth, indicating an increased requirement for the chromatin remodeling complexes to maintain self-renewal capacity in adult HSCs [200, 201]. These developmentally regulated changes in transcription factor dependency suggest a requirement for active transcriptional reprogramming to accommodate the changing demands of HSCs during ontogeny.
Table 2
Differences between E14.5 fetal and adult BM HSCs

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<th>Adult HSC</th>
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<td>Adult bone marrow</td>
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<td><strong>Protein synthesis rate</strong></td>
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<td><strong>Transcription factors</strong></td>
<td>Sox17, Lin28b</td>
<td>Gfi-1, C/EBPa</td>
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<td><strong>Metabolism</strong></td>
<td>Oxidative phosphorylation</td>
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Changing hematopoietic niches during fetal development

To understand the differences between FL and adult BM HSC states it is important to consider how the cells develop and arrive to their niche. Counter to adult hematopoiesis which takes place in the BM, in utero development of mammalian hematopoiesis occurs in spatially and temporally overlapping waves. The anatomical locations span both intra and extra embryonic tissues and adds significant complexity to the study of embryonic blood development. A brief account of the three waves of early ontogeny hematopoiesis is summarised here.

The first hematopoietic cells arise from the extra embryonic yolk sac (YS) in the primitive wave of hematopoiesis prior to the emergence of HSCs around E7. Erythroid progenitors of nucleated erythroblasts emerge and transiently proliferate in the YS until E8.5 [202] before entering circulation into the embryo proper with the onset of cardiac contraction [203]. Concomitantly with nucleated erythroblasts, primitive macrophage and megakaryocyte progenitors emerge [204]. The primary function of primitive hematopoiesis is to supply the rapidly growing embryo with oxygen carrying cells, megakaryocytes for vascular maintenance and tissue remodelling macrophages.

The second wave of YS hematopoiesis is transient and definitive arising by endothelial to hematopoietic transition (EHT). This wave first appears around E8 and is characterised by erythro-myeloid progenitors (EMPs) suggested to arise from a distinct type of hemogenic endothelium without the capacity to later generate HSCs [205]. EMPs generate tissue-resident macrophages that will sustain over the
The transition to definitive hematopoiesis in mice is marked by a switch from embryonic to adult haemoglobin in EMP derived erythrocytes. Later progenitors from the second wave emerging around E9.5 have lymphoid and myeloid potential independent of HSC input [71]. Together the yolk sac originated waves of early hematopoiesis supply the first cells of the blood system prior to the emergence of HSCs.

The third wave gives rise to definitive HSCs. HSCs are first generated at E10.5 in the aorta-gonad-mesonephros (AGM) region from hemogenic endothelium in the ventral wall of the dorsal aorta [67, 209]. Live- imaging studies have shown the generation of definitive HSCs by EHT where endothelial cells start to bulge into the lumen before rounding up and detaching into circulation [210]. At around the same time HSCs are observed in other anatomical locations of the embryo; major arteries [211] and the head [212].

During fetal development, the main function of the liver is hematopoiesis and it is the predominant niche for HSC expansion. Following their generation in the AGM HSCs seed the fetal liver from E11 [209]. Here they undergo rapid proliferation between E12-E16 [213]. The FL niche contributes to HSC maintenance through different microenvironment factors. HSCs within the fetal liver are located in close proximity to a distinctly proliferative type of Nestin^Ng2^ pericytes on portal vessels. In the absence of FL Nestin^Ng2^ cells HSC numbers decrease by ~45% [214]. Furthermore, SCF^DLK^ hepatic progenitor cells, another FL niche constituent have been shown to support culture of HSCs in vitro [215]. Finally, as previously discussed bile acids support rapid translation of protein in proliferating HSCs through alleviation of ER stress [175].

Following expansion in the FL, HSCs migrate first to the spleen at around E15.5 and start seeding the BM at E17.5 [216]. Stromal cell derived factor 1 (SDF-1) is essential for the transition from FL to BM hematopoiesis. SDF-1^-^ mice have normal numbers of FL HSCs but colonization of the BM is severely impaired [217]. HSCs reside within the vascularized regions of fetal BM, and require osteolineage cells to maintain their function [218]. Adult HSCs are primarily located in a perivascular niche within the BM, recognized for its role in maintaining adult BM HSC quiescence [219].
Figure 4
Lin28 induced fetal-like hematopoiesis

The first published demonstration of the ability of Lin28 to induce fetal-like hematopoiesis in the adult blood system came in 2012. Searching for molecular mechanisms to explain the switch from fetal to adult lymphocyte output [170, 171], the authors identified a fetal specific expression pattern of Lin28 in HSPCs, B and T cell progenitors. Importantly ectopic Lin28 expression in adult bone marrow repressed Let-7 miRNAs and reinitiated fetal-like lymphopoiesis, including the generation of innate-like B-1, MZ, iNKT and gdT cells [220]. These findings highlighted Lin28 as a molecular switch to induce fetal-like properties in adult BM HSPCs. These initial findings were extended with the identification of Hmga2 as a downstream target of Lin28b, which increases self-renewal in HSCs [221]. This study linked regulation by the Lin28b/Let-7 axis to the switch from fetal to adult HSC self-renewal state. Interestingly, while overexpression of Hmga2 phenocopies the ability of Lin28b to increase self-renewal in serial-transplantation it does not induce innate-like B lymphocyte generation. More recently, the mechanism for Lin28b induction of fetal B-lymphopoiesis was further elucidated with the identification of Arid3A as a critical downstream mediator [222]. Ectopic expression of Arid3A in adult BM pro-B cells enabled differentiation into B-1 cells, although with lower efficiency compared to Lin28b. Furthermore, the \( V_H \) usage of Lin28 induced B-1 cells was investigated and despite having the phenotype of fetal derived B-1 cells they did not harbour a typical fetal BCR repertoire. However, these studies relied on retroviral mediated constitutive overexpression of Lin28 and do not distinguish between the effect of Lin28 on the development of B-1 versus its effect on mature B-1 homeostasis. Many open questions remain in the puzzle of Lin28 induced fetal-like lymphophoiesis. Can B-1 cells induced by transient Lin28 expression replicate other functional hallmarks of fetal derived B-1 cells, as self-renewal in the periphery and natural antibody production? If so, how? (Paper II)

The balance between myeloid and erythroid progenitors is developmentally regulated to accommodate the stage-specific requirements of mature cells from each lineage. During embryogenesis the immediate need for oxygen delivery skews the
balance towards erythroid output [223], while myeloid and megakaryocyte progenitors increase with age [224] along with the risk for myeloid disease [225]. The Lin28/Let-7 axis has been shown to extent to the myeloid-erythroid progenitor balance. Lin28b overexpression in adult BM induced an increase in erythroid progenitors concomitantly with a decrease in myeloid progenitors. Oppositely, Lin28b deficiency during fetal development resulted in an adult phenotype biased towards myeloid progenitors. This demonstrate the requirement for endogenous Lin28b in regulating the myeloid-erythroid progenitor balance during early development [226]. Further cementing the influence of Lin28b on the myeloid-erythroid system; fetal-like mast cells accumulate upon Lin28b overexpression in adult BM [227] and Lin28b expression in adult erythroblasts induces the switch from adult to fetal haemoglobin correlating with a Let-7 dependent decrease in Bcl11a [228].

Recent reports have further highlighted the role of Lin28 in the differentiation of T effector cell subsets with distinct functions in early fetal and neonatal development. Immune suppressive regulatory T cells are higher in frequency in the fetus compared to the adult. In utero they modulate the immune response against maternal antigens to induce tolerance in human [230]. Upon knockdown of Lin28b in fetal naïve T cells their capacity to generate regulatory T cells is significantly diminished, illustrating Lin28b as a key mediator of the tolerogenic fetal T cell program [231]. Neonatal CD8+ T cells preferentially differentiate into short-lived effectors after infection, while adult CD8+ T cells can contribute to the long-lived memory pool.
Upon Lin28b induction, adult CD8^+ T cells gain a short-lived neonate-like effector phenotype [232].

Together, these studies on Lin28 induced fetal–like hematopoiesis establish Lin28 as a key player in orchestrating the developmental switch from fetal to adult hematopoiesis in the erythroid, myeloid and lymphoid arms of the blood and immune system.
Thesis aims

My thesis work has focused on understanding the developmental switch from fetal to adult B lymphopoiesis.

The specific aims were the following:

- To investigate the cellular basis for the postnatal attenuation of B-1a potential (Paper I and IV).
- To assess the role of Lin28b in potentiating positive selection of self-reactive B-1a cells (Paper II)
- To compare chromatin accessibility changes between E14.5 FL and adult BM HSPCs (Paper III)
Summaries of included papers

Paper I and IV

At the time that this thesis project was initiated in 2014, the decades long debate on B-1a origins and development had taken a new turn. Led by new findings in the laboratory of Prof Leonore Herzenberg (Stanford University), the idea emerged that B-1a cells may not share a common hematopoietic progenitor with B-2 cells at all and instead exclusively derive from a transient wave of embryonic progenitors that arise prior to and independently from definitive HSCs (B-1 Cell development and Function, Merinoff Congress 2014 NY). This scenario was built upon the existing ‘Lineage model’ and provided an attractive explanation for the developmental attenuation of B-1a cell output which was not addressed by the original Lineage vs Selection model debate. It found support in the now widely acknowledged existence of non self-renewing primitive progenitors harboring B-1a cell potential without generating B-2 cells [70, 233]. However, the negative data presented at the B-1 congress and later published in Stem Cell reports [73], showing that a highly purified FL HSC population failed to generate detectable B-1a cell output, did not in our opinion preclude the previous notion that HSCs are the major source of B-1a cells later during ontogeny [95, 171, 234]. Furthermore, it does not address the body of evidence supporting the Selection model and is in direct opposition to the body of evidence demonstrating a low yet steady level of adult bone marrow dependent contribution to the B-1a cell pool [20, 77]. We reasoned that the attenuation of B-1a output could be caused by a decrease in HSC B-lineage plasticity after birth, and not solely by the loss of fetal HSC-independent B-1a restricted progenitors (Figure 6). Several questions needed to be answered to assess the cellular basis for the postnatal decrease in B-1a output. First, do B-1a and B-2 cells share a common hematopoietic progenitor? Second, can functionally defined HSCs in the FL generate both cell types at the single cell level? These questions cannot be addressed by traditional population-based studies. Instead, the prerequisite ability to track single cell fates in vivo while enabling functional rather than immunophenotypic HSC definition must be met.
Around the same time, lentiviral cellular barcoding had come of age, quickly becoming a state-of-the-art method for long-term tracing of single HSPC fates in vivo [235, 236]. Previously, in-vivo clonal lineage tracing had been tackled by transferring single congenially marked HSCs into recipient mice [237]. However, the disputed origin of B-1a progenitors along with the obvious time and cost inefficiency of single cell transplantations made it an undesirable approach. Considering both the strengths and limitations of cellular barcoding we realized its unique potential in providing well-timed insight into the developmental relationship between B-1a and B-2 cells at the level of multipotent HSPCs (Figure 7).
First, cellular barcoding allows for the simultaneous tracking of hundreds of individual cell fates in a single transplanted animal, which means that a heterogeneous group of cells can be targeted and traced in vivo. Second, with cellular barcoding HSCs can be defined by function rather than immunophenotype. Lineage output can be traced from multiple HSPC clones across serial transplantation enabling retrospective identification of HSC clones, without requiring a pure HSC donor population. An important limitation of the cellular barcoding method, often overlooked, is that while it can establish the presence of potential, it can never be used to establish the lack of potential [238]. There are many possible explanations for the lack of detectable output at the time of analysis, including detection sensitivity and turnover rates of the founder and mature cell types. In this respect, we benefit from HSCs and B-1a cells both being long-lived, self-renewing cell types with a slow turnover rate. These characteristics are highly suitable for long-term tracing and maximize the chances of a positive readout in establishing a direct link between developmental changes in HSC behaviour and B-1a potential at the single cell level. Another limitation of cellular barcoding is related to the complexity of the barcoding library. Less complexity in a library increases the risk of the same barcode integrating in multiple cells. This risk increases with the number of labeled cells. However the high reconstitution potential of FL HSPCs enables efficient readout from relatively low numbers of labeled cells. A last complication of cellular barcoding is the risk of multiple integrations in the same cell. However, in resolving the lineage relationship between B-1a and B-2 cells, our most important readout is barcode overlap.

Taken together, these compelling arguments led us to embark on establishing cellular barcoding in our own lab to shed light on the developmental origins on B-1a cells. Since cellular barcoding had at the time never been performed on FL HSPCs, we invested significant amounts of time and energy to optimize this protocol (Paper IV). Cellular barcoding allowed us to demonstrate that functionally defined FL definitive HSCs give rise to both B-1a and B-2 B cells at the single cell level. Initially B-1a potent HSCs become B-2 restricted upon secondary transplantation consistent with a developmental decrease in lineage plasticity. Our results not only disproved the notion that definitive HSCs lack B-1a potential [66], but establish a direct link between the attenuation of B-1a potential to developmental changes in HSC behaviour. Furthermore, we show that B-1a potential can be reinitiated by Lin28b expression in a polyclonal fashion that coincides with the reversal to a fetal like HSC state, characterized by increased self-renewal and repopulation potential.
*Paper I* made clear that developmentally restricted HSPC origins alone cannot account for the developmental attenuation of B-1a cells, setting the stage for further examination of the basis for this developmental phenomenon. To this end, our lab took two parallel approaches. First, we investigated whether developmental and Lin28b induced changes in repertoire selection could selectively potentiate B-1a cell selection into the mature B cell pool early in life (*Paper II*). Second, we investigated developmental changes in HSC and LMPP chromatin accessibility to explore regulation of a fetal HSC state (*Paper III*).
The peripheral lymphocyte pool is largely shaped by constraints in repertoire selection. Thus, it is conceivable that changes in BCR selection criteria may contribute to the developmental attenuation of B-1a cells, such that a typical B-1a BCR would be allowed to mature early in life but exceed the permitted threshold for B cell tolerance in the adult. Several lines of evidence suggest a more lenient threshold for BCR repertoire selection during fetal and neonatal life. First, while developing B-2 cells in the adult initiate central tolerance mechanisms to eliminate self-reactive specificities, B-1 cells become positively selected in a self-antigen dependent manner [79]. Second, non-conventional heavy chains that pairs poorly with SLC are selected into the fetal / neonatal but not adult B cell repertoire [82, 84]. Third, work using several B-1 specific BCR transgenic models demonstrated self-antigen dependent positive selection into the mature B cell pool during neonatal life but tolerance induction and exclusion from the mature B cell pool in adult life [84, 239]. Fourth, T cell repertoire selection in neonates has been shown to be skewed towards clonotypes with higher self-MHC-peptide affinity compared to adults, skewing the neonatal T cell repertoire towards self-reactivity [103]. Despite these clues, the use of antigen receptor transgenic models have made it difficult to uncouple global changes in repertoire selection from B-1 specific BCR instructed fates.

In 2015, published data from the Hardy lab [222] as well as our own unpublished data had made clear that ectopic expression of Lin28b in adult proB cells was sufficient to potentiate fetal-like B-1a cell output. This led us to hypothesize that Lin28b may play an important role during the latter part of B lymphopoiesis to potentiate the positive selection of B-1a cells early in life. In Paper II, we show that CD5 levels of B-1 cells are developmentally set in the immature B cell stage and correlates with self-reactivity similar to what has been observed in the T cell lineage. Genetic perturbation studies show that Lin28b is necessary and sufficient for efficient positive selection of CD5+ B cells and potentiates neonatal immature B cell CD5 expression in a dose dependent fashion. Importantly, our results are generated in a WT polyclonal BCR setting and uncouples positive selection from specific BCR identities, implicating the heterochronic RNA-binding protein as the missing link that regulates the developmental attenuation in B-1a cell output through relaxing the permissiveness of B cell selection. Our findings shed light on the unique ability of B-1a cells to escape tolerance and undergo T cell like positive selection and are consistent with an evolutionary benefit in the developmental enrichment for useful specificities in the T independent arm of the B cell lineage.
In Paper I we identified a link between a fetal HSC state and B-1a potential. The ability of Lin28b to induce a fetal-like HSC state in adult BM HSCs raised the question of how such a state would be regulated and what the underlying mechanism might be. The clear heterogeneity within the BM HSC compartment in terms of induced B-1a potential upon Lin28b overexpression hint at a set layer of regulation that Lin28b overexpression cannot overcome. Furthermore, the increased HSC self-renewal observed upon ectopic expression of Lin28b (Paper I and [221]) made us speculate about a possible stress alleviating role of this post-transcriptional regulator. We initiated this project to explore the levels of regulation enabling the fetal HSC state.

The preliminary work in article III is part of ongoing studies ultimately aimed at understanding how Lin28b contributes to a fetal hematopoietic stem cell state. The results in this manuscript compare the accessible chromatin state of E14.5 FL and adult BM HSPCs by assay for transposase-accessible chromatin (ATAC-seq) [240].

In agreement with a more complex proteome in adult HSPCs compared to FL [176] we found a generally more accessible chromatin landscape in adult BM HSCs and LMPPs. Interestingly, transcripts that are more abundant in FL HSCs are uncoupled from unique FL chromatin signatures, while transcripts more abundant in ABM HSCs closely correlate with open associated chromatin regions. This indicates a higher reliance on post-transcriptional regulation during early life hematopoiesis and is consistent with the fetal specific expression pattern of the post-transcriptional regulator Lin28b.
General discussion and future directions

Our findings in Paper I showing B-1a output from fetal HSCs are in direct opposition to the conclusions of a recent report stating that FL HSCs lack B-1a potential [73]. The results however can perfectly coexist. Indeed, we identified multiple FL HSC clones without contribution to the B-1a population. While we cannot rule out that lack of detection is linked to the sensitivity of the cellular barcoding method, the alternative explanation of heterogeneity within the HSC compartment is a likely reason (discussed further in next section). This however does not extend to the hypothesized B-1a restricted HSC [234]. In our study we did not detect FL HSC clones contributing to the B-1a compartment without also giving rise to B-2 cells.

Following our findings in Paper I independent lines of study have corroborated the identification of fetal hematopoietic progenitors with shared B-1a and B-2 B cell potential at single cell resolution. The Bernstein group used a clonal assay to show that single E11.5 AGM pre-HSCs had the capacity to generate both B cell subsets [241]. The barcoding polylox model generated by Rodewald lab showed that during unperturbed hematopoiesis Tie2-cre labeling at E9.5 HSC generated HSC clones that in the adult mouse had contribution to both B-1a and B-2 subsets [242].

Collectively our study showed that attenuation of B-1a potential after birth is explained by a shift in HSC potential and not solely by the loss of B-1a restricted FL progenitors. It remains to be shown if B-1a cells generated prior to HSC emergence contribute significantly to the adult B-1 compartment in situ (Figure 8). Future studies utilizing inducible B cell fate mapping models could conclusively answer this long-standing question. Furthermore, in situ labeling would enable functional comparisons between adult derived and early life derived B-1 cells to elucidate their roles in the adult B cell compartment.

Lin28b is acknowledged as a key player in inducing fetal hematopoiesis. However, the underlying mechanism enabling Lin28b induced fetal-like B-1 lymphopoiesis remains unclear. Is this mechanism only going through the Arid3A Let-7 dependent
mechanism [222] or is the mRNA interactome of Lin28b affecting B-1 development? Future studies will investigate this line of questions.

Figure 8
The influence of developmental timing on B cell diversity. A schematic model of how the adult B cell repertoire is shaped by developmental changes in B cell output. During ontogeny, the first primitive wave of hematopoiesis lacks detectable lymphoid potential. The second wave is initiated prior to the emergence of HSCs and contains hematopoietic progenitor cells (HPCs) with lymphoid potential. Subsequently, HSCs become the source of lifelong hematopoiesis. Initially, mouse FL HSCs can give rise to both B-1a and follicular B-2 B cells at the clonal level. Interestingly, FL HSCs appear to be heterogeneous in their ability to contribute to the long-lived B-1a cell pool. The output of self-reactive B-1a cells declines after birth, at least in part, due to a developmental attenuation of Lin28b expression. B-1a cells in adult mice are maintained long-term in a self-antigen driven manner. The absence of Dntt expression in fetal and neonatal life favors homology directed recombination events and allows for the selection of useful self-reactive specificities while minimizing the risk for the output of B cells with harmful autoimmune specificities. Illustration from [243].
HSC heterogeneity

HSC heterogeneity has mostly been studied in the adult BM HSC compartment with a focus on lineage contribution and reconstitution. Examples of surface marker intensity defining HSC subsets are Sca-1 [244] and CD150 [245] which have been shown to influence both long-term reconstitution capacity and the balance between myeloid and lymphoid output. The identification of platelet biased HSCs residing at the top of the hematopoietic hierarchy and their contribution in unperturbed hematopoiesis has further broadened the concept of HSC heterogeneity [246, 247]. Fewer studies have explored HSC heterogeneity during fetal development. A comprehensive single cell transplantation study was performed by Eaves laboratory to investigate HSC heterogeneity from E14.5 FL to 2-year-old BM. They reported an increase in myeloid biased HSCs already in the transition from FL to fetal BM which subsequently increased with age [237]. An interesting outcome of Paper I is the observed HSC heterogeneity with respect to B-1a cell potential. While Lin28b expression was detected in virtually all E14.5 FL HSCs this did not translate to homogeneity in lineage output where a bit under 50% of the clones had B-1a output detected. While not directly comparable to the fetal BM, we did observe a decrease in this fraction of HSCs with B-1a potential when we traced barcoded newborn BM LSKs perhaps mirroring the increase in myeloid biased HSCs observed by Eaves. Our comparison of barcoded adult BM LSKs with and without Lin28b overexpression provides an equal starting point to interrogate the effect of Lin28. This experiment showed a clear heterogeneity in the ability of Lin28 to induce fetal-like lymphopoiesis. Lin28b is expressed in all cells in the tet-Lin28b mouse model but only 1/3 of the adult HSC clones generated B-1a cells, demonstrating heterogeneity within the adult HSC compartment. While Lin28b induced self-renewal in the HSC compartment previously had been demonstrated [221], the clonal resolution in our study suggests that the increase in phenotypic HSC frequency is largely due to an increase in the HSC subtype which has the capacity to generate B-1a. The reproducible pattern with approximately 1/3 of HSC clones contributing to B-1a cells upon Lin28b overexpression suggests that this potential is somehow hardwired at the HSC stage and is passed on to downstream progenitors. What enables this subset of HSCs to generate B-1a cells? And what distinguishes them from other HSCs in the compartment? Future studies will be aimed at linking this upstream heterogeneity with generation of self-reactive B cells. This could elucidate an impact of HSC heterogeneity on immune function.

Recently it has been suggested that a developmentally restricted subset of HSCs only contribute to hematopoiesis early in development but not during adult life
This study identified a developmentally restricted Flt3 expressing HSC biased toward innate-lymphocyte output. This transient HSC-type met the criteria of serial transplantability, but disappeared from the HSC compartment shortly after birth. A transient wave of HSCs in early neonatal life could serve to rapidly generate a layer of mature immune cells for immediate protection [248]. While these HSCs are most likely represented within our FL barcoding experiments, their low frequency would suggest a negligible impact on the overall distribution of clones. Furthermore, their low myeloid output separate them from B-1a generating HSC clones in our experiment which contributed to the majority of granulocyte output.

The fetal HSC paradox – a role for Lin28?

The high proliferation rate of fetal HSCs is associated with high reconstitution capacity in transplantation assays while the opposite is true for adult HSCs. This is paradoxical and suggest that fetal HSCs must have developed a way to overcome the stress associated with high rate of replication and a metabolism with high production of ROS while simultaneously retaining higher functionality than their adult counterparts. We reason that two possible explanations are at play. Either HSCs in the FL are alleviated from stress through factors that are lowly expressed in adult HSCs, or they may have a mechanism to ignore the stress response.

In Paper III we found a disconnect between gene expression and chromatin state in FL HSCs which was not observed for adult HSCs. This disconnect suggests that genes with high transcript levels in FL HSCs compared to adult BM HSCs rely on post-transcriptional regulation for their expression. Among the genes higher
expressed in FL HSCs but not associated with differences in chromatin accessibility were several DNA damage repair proteins. Interestingly, several of these proteins are reported Let-7 targets; Chek1, Rrm1, Rrm2, Mcm2-10, Fancd2 and Brca1/2 [134]. This implicates Lin28 as a putative candidate for enabling the higher fetal expression of these genes in HSCs. Studies have implicated these DNA damage repair factors in alleviation of replication stress [249, 250] and some are essential for FL hematopoiesis and development. Deficiency of mini-chromosome maintenance complex (MCM) 3 was shown to cause replication stress and embryonic lethality characterized by anemia and impaired HSC fitness [251]. Fancd2<sup>−/−</sup> embryos show significant defects in fetal liver hematopoiesis with specific decrease in HSC numbers and increased HSC apoptosis leading to anemia and bone marrow failure in adult mice [252]. Additional evidence for a stress alleviating mechanism of Lin28 comes from the observation that overexpression in cell lines confers radiation and chemotherapy resistance while increasing cell proliferation and decreasing apoptosis [253, 254]. Future studies will advance this preliminary concept put forth in Paper III to uncover a possible role for Lin28 in FL HSC stress alleviation.
Genetiske stregkoder viser immuncellers slægtskab.


I voksenlivet bliver de mature blodceller produceret fra blodstamcellerne i knoglemarven. Blodstamcellerne forbliver hele livet igennem på et umodent/udifferentieret stadium. Deres regenerative potentiale er eksemplificeret i knoglemarvstransplantationer, hvor blodstamceller fra donor danner et helt nyt system i recipienten. For at danne nye mature blodceller gennemgår blodstamcellerne mange mellemstadier (progenitorceller), hvor de bliver tiltagende specificeret. Man kan beskrive denne proces som et hierarki, hvor stamcellen i toppen er ophav til alle de mature celler i bunden. Da jeg påbegyndte mit PhD projekt, var der stor kontrovers inden for B-1 celle feltet. En ny teori postulerede, at B-1 celler ikke kunne genereres fra blodstamceller. Denne teori anbragte i stedet B-1 celler i et separat udviklingssystem, som var helt uafhængigt af blodstamceller og antog, at B-1 cellerne aldrig delte et udviklingsstadium i blodsystemet med B-2 cellerne. Der var flere problemer med denne nye teori, men for at modbevise den, måtte vi kunne vise slægtskabet mellem de mature celler og deres progenitorceller. Vi havde brug for en metode, der kunne skelne mellem enkelte donorcells bidrag til mature cellegrupper. En sådan metode var for nylig udviklet; ved at inkorporere en unik genetisk ”stregkode” i DNAet på stamceller, vil denne blive nedarvet til alle
blodceller, der er dannet fra samme stamcelle. Ved at stregkode blodstam- og progenitorcellerne og derefter transplantere dem i mus, kunne vi vise, at B-1 og B-2 celler kan genereres fra en progenitorcelle i fosterstadiet, og at blandt andet blodstamceller har denne evne.


Blodstamcellerne i fosteret deler flere karakteristika med cancerceller. De deler sig ofte, migrerer mellem forskellige anatomiske lokaliteter, og udtrykker mange gener forbundet med vækst. Dette er i modsætning til voksne blodstamceller, som er i dvale det meste af tiden, og som har et lavt bioenergetisk niveau. Lin28, som er udtrykt specifikt i fosterudviklingen, er også udtrykt i flere typer cancer. For at forstå sammenhængen mellem Lin28 og fosterblodstamcellernes unikke egenskaber, er vi begyndt at undersøge DNA konfigurationen af disse.

Samlet har dette PhD projekt identificeret cellulære og molekylære mekanismer i regulering af overgangen mellem føtal og voksen B cellesgenerering.
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References


Switching ON Fetal B Lymphopoiesis

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Science-covered chocolate